EXHIBIT 4

Gene transfer to stomach mucosa of mice by oral administration of chitosan-packaged DNA polyplex.

This study was conducted to demonstrate that chitosan-packaged plasmid DNA delivered orally to mice could lead to gene transfer to and gene expression in mucosal cells of stomach. A plasmid carrying the GIP promoter-linked human insulin gene construct or a E1Fα promoter linked SEAP gene was mixed with a chitosan (MW: 3.8 kD, Degree of deacetylation = 98%) at a chitosan:DNA ratio of 40:1. The polyplex was packaged and characterized according to previously described methods.

Overnight fasted mice were fed 0.5 mL of a suspension containing chitosan-packaged DNA polyplex (at [DNA] = 75 μg/ml) in a single bolus via a feeding tube. Four hours after vector delivery, treated animals were returned to their cages with free access to food and water. Two days after oral feeding of the polyplex, animals were sacrificed and stomach mucosa collected by tissue scrapping. The levels of insulin or SEAP mRNA were quantified by a standard reverse-transcription real-time quantitative PCR assay.

As show below in Figure 1, a single oral administration of chitosan-packaged DNA particles results in transformation and expression of insulin and SEAP gene in stomach mucosa. Therefore, transformation of cells in stomach can be achieved by oral administration of a vector without undue experimentation.

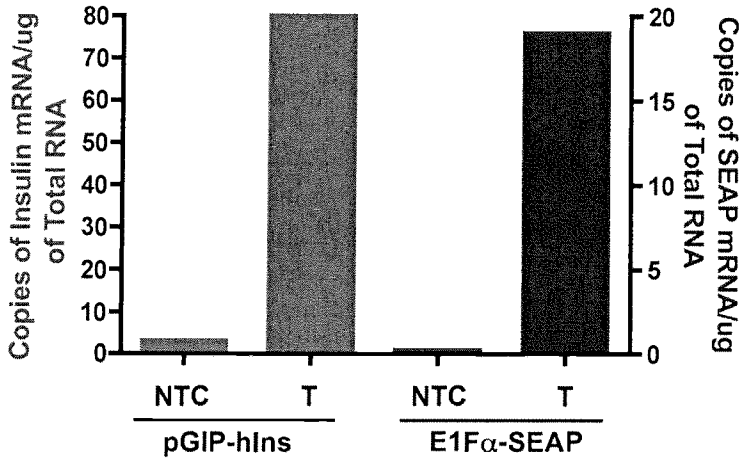

Figure 1 : Transformation of stomach mucosal cells by oral delivery of a chitosan-packaged DNA polyplex. Levels of insulin (red) and SEAP (blue) gene expression levels in the stomach mucosa of mice at 2 days after oral administration of vector. NTC=non-treated controls, T= treated animals.